Journal of Cell Science

ADAR1 inhibits adipogenesis and obesity by interacting with Dicer to promote the maturation of miR-155-5P

Zuying Yu, Ruijie Luo, Yutian Li, Xiaoguang Li, Zhengrui Yang, Jiangtong Peng and Kai Huang

DOI: 10.1242/jcs.259333

Editor: James Olzmann

Review timeline

Original submission:	31 August 2021
Editorial decision:	19 October 2021
First revision received:	7 January 2022
Accepted:	8 January 2022

Original submission

First decision letter

MS ID#: JOCES/2021/259333

MS TITLE: ADAR1 inhibits adipogenic differentiation and high fat diet-induced obesity by interacting with dicer to promote the maturation of miR-155-5P

AUTHORS: Zuying Yu, Ruijie Luo, Yutian Li, Xiaoguang Li, Jiangtong Peng, and Kai Huang
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to

all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The current studies identify ADAR1 as an inhibitor of adipogenesis via enhanced production of miR-155-5p and downregulating the adipogenic early transcription factor C/EBPβ.

Comments for the author

The studies in this manuscript features the role of ADAR1 as an inhibitor of adipogenesis via enhanced production of miR-155-5p and downregulating the adipogenic early transcription factor C/EBPβ.
They report that both mRNA and protein levels of ADAR1 were downregulated significantly in inguinal white adipose tissue (iWAT) from obese mice The key findings are:
1. The expression of ADAR1 declines during adipogenesis and decreases in obese iWAT.
2. Overexpression of ADAR1 inhibits adipogenic differentiation and knockdown of ADAR1 promotes adipocyte differentiation
3. Overexpression of ADAR1 inhibits HFD induced obesity
4. ADAR1 controls the expression of late TFs by regulating C/EBPβ
5. ADAR1 interacts with Dicer that promotes the maturation of miR-155-5p
In vitro studies are well-designed and robust in support of the inhibitory role of ADAR1 in adipogenesis.
However, anti-adipogenic function of ADAR1 cannot explain the in vivo phenotype when Adar1 is targeted by AAV. If ADAR1 is purely anti-adipogenic, the KD mice will be lipodystrophic and metabolically unhealthy. The authors should explore other possibilities that can explain for in vivo phenotype, such as altered energy homeostasis. In fact, a recent study (PMID: 33252250) showed that Adar1+/- mice display a similar phenotype due to reduced food intake. The fact that ADAR1 is reduced in iWAT may suggest that it is involved in beige adipogenesis.

Major points:
1. What is the cell autonomous effects on insulin sensitivity?
2. Is there altered energy balance and body composition in the shAdar1-treated mice?
3. In Fig. 7, analogous studies are necessary by knocking down miR-155 to test the necessary role in ADAR1-mediated anti-adipogenesis.

Minor point:
There are 2 bands in ADRB1 western blotting. Are they two different isoforms?

Reviewer 2

Advance summary and potential significance to field

Although the interaction between ADAR1 and Dicer, Dicer and miR-155 (miRNA in general) and miR-155 and CEBPb are already known, they showed a potential mechanism as to how ADAR1 could inhibit adipogenesis both in vitro and in vivo which was not addressed in an existing publication "ADAR1 deficiency protects against high-fat diet-induced obesity and insulin resistance in mice" (doi:10.1152/ajpendo.00175.2020).

Comments for the author

Major comments:
[Fig1A] It is unclear if showing ADAR1 tissue expression could be beneficial especially since it is very high in the brain.
[Fig5] Please clarify how the reduced WAT mass by ad-ADAR1 injection improves GTT and ITT as adipose tissue can promote glucose clearance from the blood.

Similarly, what would be the potential mechanisms where ad-ADAR1 injection could reduce the serum TAG and cholesterol levels.
It would be recommended to include and discuss a similar study done in the field, "ADAR1 deficiency protects against high-fat diet-induced obesity and insulin resistance in mice" (doi:10.1152/ajpendo.00175.2020).

Minor comments:
1. Please indicate that NC stands for negative control in the text and/or legends.
2. Please use the consistent, standard format for gene/protein names throughout the manuscript. The same gene is sometimes all capitalized and sometimes not.
For example, PPARg vs Pparg. CEBPb vs C/EBPb vs CEBP/b. etc.
3. [The paragraph of ADAR1 overexpression] It is conventional to use the word "transduction" for virus instead of transfection.
4. [Fig5 legend] Please correct "...andmethod".
5. [Fig5 legend] It could be misleading to call adipoq and fabp4 adipogenic genes since they are terminal genes and not the driving factors for adipogenesis.
6. [Fig7A] Pleas indicate which band is the target of interest and which more than one band is present.

First revision

Author response to reviewers' comments

RESPONSES TO PEER REVIEWER COMMENTS

Dear editor-in-chief, associate editors, and reviewers,

We are very grateful to have the opportunity to improve our manuscript (JOCES/2021/259333) entitled “ADAR1 inhibits adipogenesis and obesity by interacting with dicer to promote the maturation of miR-155-5P” for Journal of Cell Science. We have revised the manuscript carefully by following the comments from reviewers. The original comments are in *italics*. Our response to each comment is shown below and the revised portion in this version are marked in red.

We have carefully considered your comments as well as those offered by the two reviewers and have made in-depth and throughout revision to address **all** of them. Herein, we explain how we revised the paper based on those comments and recommendations. We would like to extend our appreciations to you for taking your valuable time and effort to provide such insightful guidance for us.

In this version, we made the following changes:

- Changed the title of this paper to “ADAR1 inhibits adipogenesis and obesity by interacting with dicer to promote the maturation of miR-155-5P”;
- Added Fig. 6 to show that knockdown of ADAR1 exacerbates HFD-induced obesity;
- Added the explanation that knockdown of ADAR1 exacerbates HFD-induced obesity in Page 6;
- Modified the discussion of this paper;
- Added Fig.S3 in the supplementary materials;
- Made some minor changes (such as for typos and mistakes) according to the reviewers' suggestions;
- Amended linguistic expressions of the full text, such as incorrect spelling, grammatical errors, inappropriate descriptions, etc.

In addition, the authors thank all the editors for their hard work on this manuscript during the covid-19 epidemic. We have made a comprehensive revision of this manuscript and hope the modifications can be approved. We will try our best to cooperate with you to revise and edit this manuscript. Wish you good health and good luck!

In the following, we offer the detailed response to each comment and concern proposed by reviewers. We hope the revised manuscript can be accepted for publication in the *Journal of Cell Science*.

Reviewer #1:

Comments for the Author:

The studies in this manuscript features the role of ADAR1 as an inhibitor of adipogenesis via enhanced production of miR-155-5p and downregulating the adipogenic early transcription factor C/EBPβ.

They report that both mRNA and protein levels of ADAR1 were downregulated significantly in inguinal white adipose tissue (iWAT) from obese mice.

The key findings are:

1. The expression of ADAR1 declines during adipogenesis and decreases in obese iWAT.

2. Overexpression of ADAR1 inhibits adipogenic differentiation and knockdown of ADAR1 promotes adipocyte differentiation

3. Overexpression of ADAR1 inhibits HFD induced obesity

4. ADAR1 controls the expression of late TFs by regulating C/EBPβ

5. ADAR1 interacts with Dicer that promotes the maturation of miR-155-5p

Response:
Thank you very much for your positive comments that provide us an opportunity to improve this manuscript. We are grateful for your valuable time and effort spent for review. Herein, we explain how to revise the paper based on your comments and suggestions. We would like to extend our appreciations to you for taking your valuable time and effort to offer such insightful guidance for us.

Major points:

1. What is the cell autonomous effects on insulin sensitivity?

Response:

Thank you for your review.

To answer this question, additional experiment were performed to evaluate the effect of ADAR1 on insulin signaling. As the results shown in Fig.S3, gene silencing of ADAR1 significantly reduced Akt phosphorylation in MEF adipocytes upon insulin stimulation. To the contrary, upregulation of ADAR1 promoted Akt phosphorylation.These data suggest that ADAR1 could activate insulin signaling pathway in cell autonomous fashion.

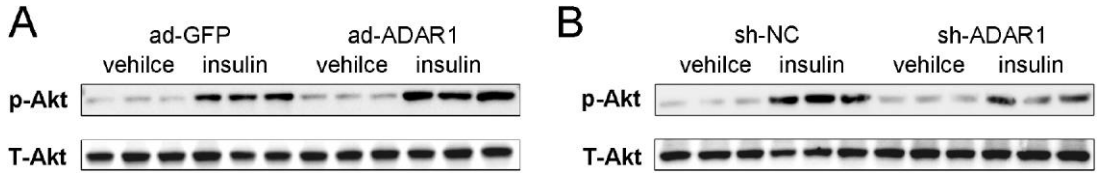

Figure S3 The involvement of ADAR1 in insulin sensitivity is adipocyte autonomous.

2. Is there altered energy balance and body composition in the shAdar1-treated mice?

Response:

Thank you for your review.

Similar to the ad-ADAR1 *in vivo* experimental design, adenovirus encoding shADAR1 was injected into the iWAT of WT mice to knockdown ADAR1 gene expression *in vivo*, followed by HFD feeding for 8 weeks. Energy expenditure was then measured by indirect calorimetry. As the result shown in Fig. 6, shADAR1-treated mice exhibited significant decrease in oxygen consumption rate(VO2) and carbon dioxide production rate (VCO2), when compared to shNC control mice. Furthermore, this was accompanied by increased body weight and fat depots on shADAR1-mice after HFD feeding.

Hope our explanation can answer your query. Thanks a lot.

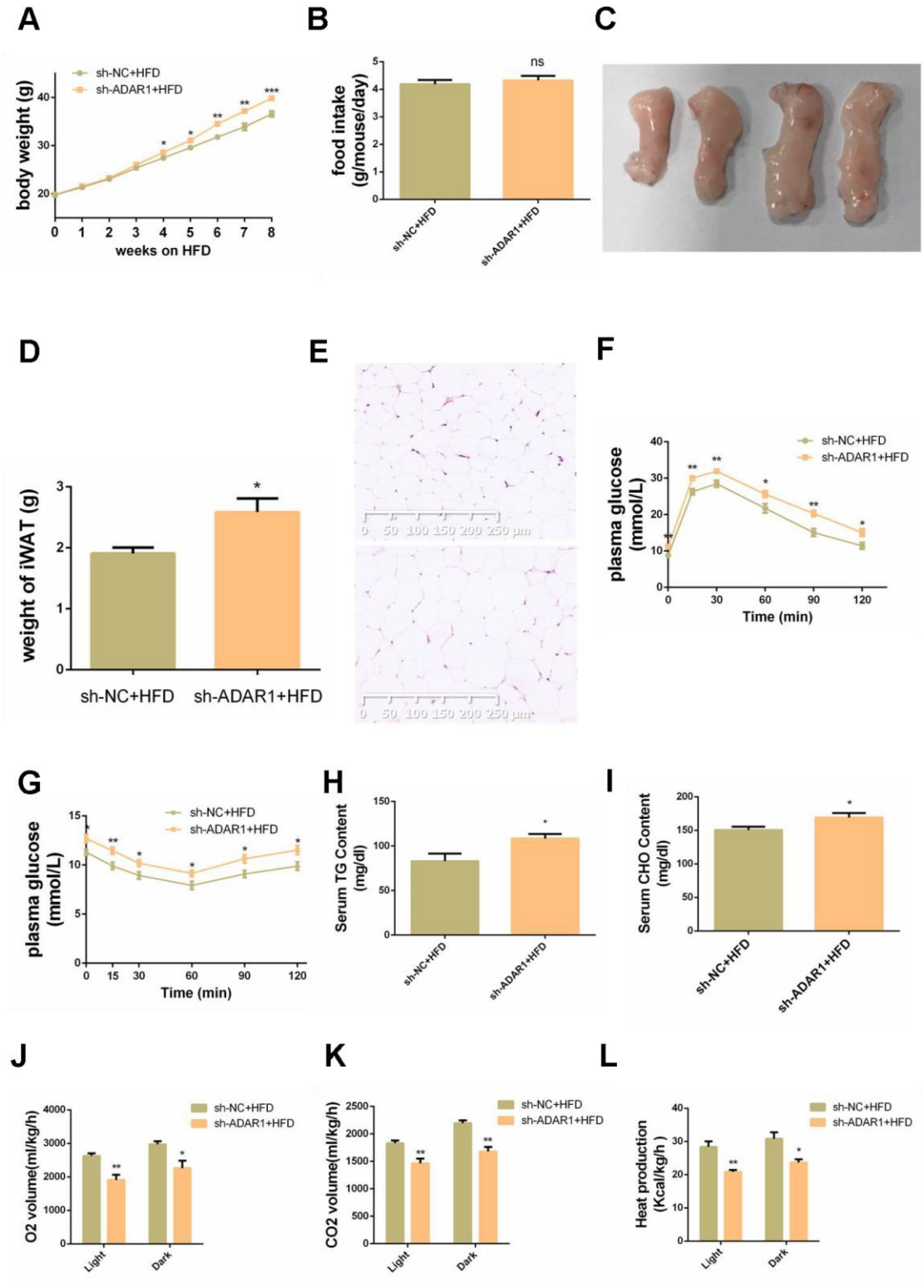

Figure 6: Knockdown of ADAR1 aggravates HFD-induced obesity.

3. In Fig. 7, analogous studies are necessary by knocking down miR-155 to test the necessary role in ADAR1-mediated anti-adipogenesis.

Response:

Thank you for your valuable suggestion.

To study the necessity of the miR-155 on the anti-adipogenesis effect of ADAR1, we treated ad-ADAR1 transduced MEFs with miR-155-5p inhibitor before induction of differentiation. As the results shown in Fig. S2, inhibition of miR155-5p could restore the expression of adipogenesis-associated genes, Adipoq, Fabp4, C/EBPα and PPARγ, which were downregulated by ADAR1.

Hope our explanation can get your approval. Thank you very much.

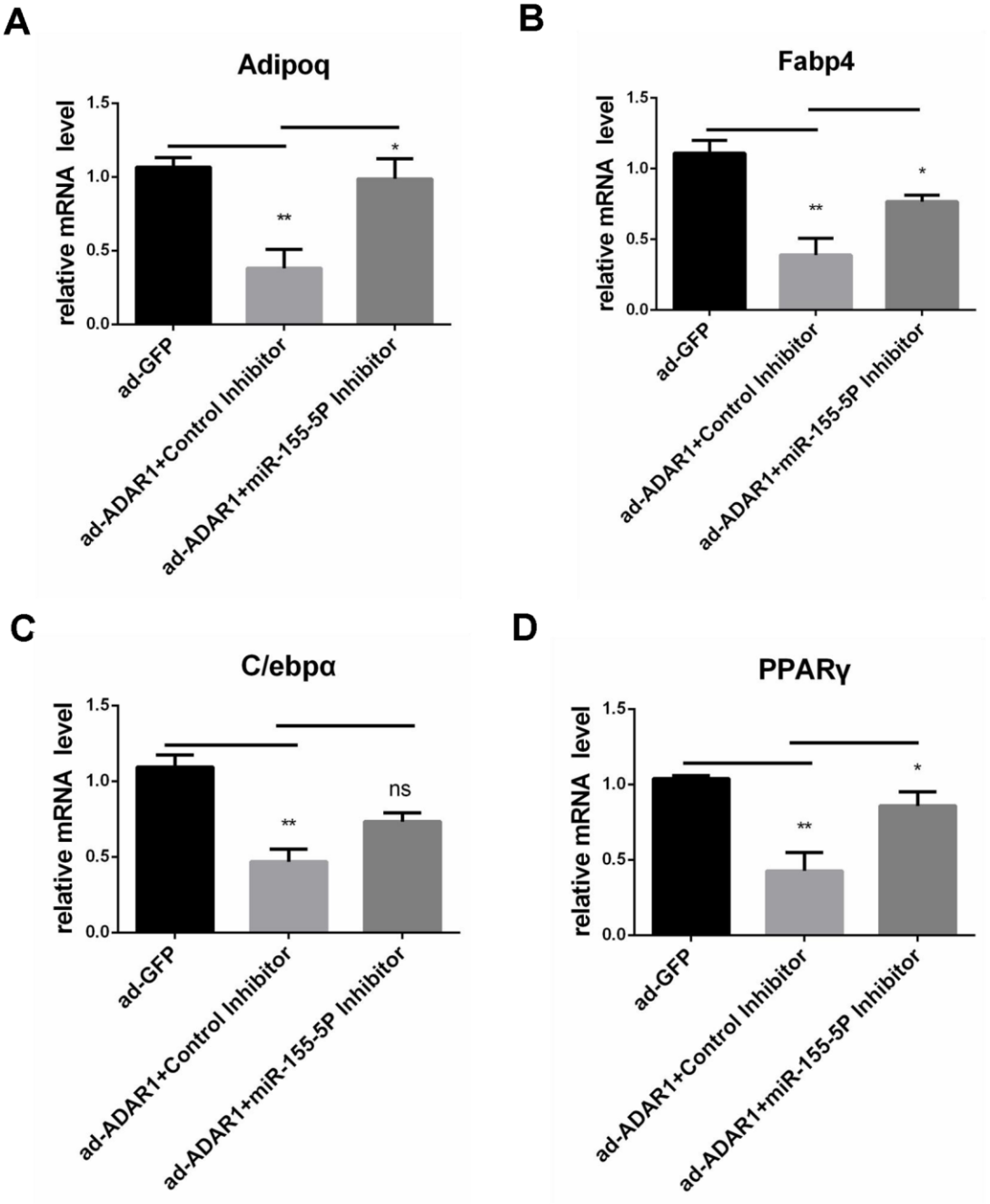

Figure S2: The inhibitory effect of overexpression of ADAR1 on adipogenic genes can be partly reversed by miR-155-5P inhibitor.

Minor point:

There are 2 bands in ADRB1 western blotting. Are they two different isoforms?

Response:

Thank you for your careful review.

ADAR1 is ubiquitously expressed with two known isoforms ADAR1L (p150) and ADAR1S (p110) resulting from transcription using alternative promoters and start codons.

Finally, all the authors sincerely thank you again, for you to give us the opportunity to improve our paper, and for your careful review. We have carefully made corresponding changes based on your suggestions. We believe that the revised manuscript can meet your requirements and will be supported to publish by you. Thank you very much!

Reviewer #2:

Comments for the Author:

Major comments:

[Fig1A] It is unclear if showing ADAR1 tissue expression could be beneficial, especially since it is very high in the brain.

Response:

Thank you for the comment. We have moved this figure to the supplementary material. Please refer to the Fig. S1 in the supplementary material.

[Fig5] Please clarify how the reduced WAT mass by ad-ADAR1 injection improves GTT and ITT as adipose tissue can promote glucose clearance from the blood. Similarly, what would be the potential mechanisms where ad-ADAR1 injection could reduce the serum TAG and cholesterol levels?

Response:

Thanks for this constructive comment. Healthy adipose tissue could promote glucose clearance from the blood, although this is not the major contributor to the direct regulation of whole-body insulin sensitivity. However, when the lipid storage capacity is exceeded as seen in our HFD feeding mouse model, adipose tissue become dysfunctional and oftentimes pathogenic. This would have large impact on the local and systemic insulin sensitivity. Under such pathogenic condition (obesity and diabetes), reducing the expansion of adipose tissue would improve glucose tolerance and insulin sensitivity, probably through decreased inflammatory responses, lower levels of adipokines and cytokines, etc.

In our manuscript, we showed that ADAR1 could downregulate C/EBPβ through increasing the production of miR-155-5p. Previous study has already shown that C/EBPβ deficiency causes reduced expression of SCD1, FASN and upregulation of CYP7A1, LXR, and ABCG1, these genes were important for the regulation of TG and cholesterol levels (Ref. 1). In addition, ADAR1 could affect energy expenditure (new data on Fig. 6), this might also have effects on TG and cholesterol levels.

Reference:

1. Rahman SM, Baquero KC, Choudhury M, Janssen RC, de la Houssaye BA, Sun M, Miyazaki-Anzai S, Wang S, Moustaid-Moussa N, Miyazaki M, Friedman JE. C/EBPβ in bone marrow is essential for diet induced inflammation, cholesterol balance, and atherosclerosis. Atherosclerosis. 2016 Jul; 250:172-9.

It would be recommended to include and discuss a similar study done in the field, "ADAR1 deficiency protects against high-fat diet-induced obesity and insulin resistance in mice" (doi:10.1152/ajpendo.00175.2020).

Response:

Thank you for your comments.We have added a discussion of this article in the final discussion section based on your suggestion.The content is as follows: In comparison, knocking down ADAR1 in adipose tissue elicited substantial impact in adipose tissue expansion, as evidenced by significantly larger adipose depots, which leads to exacerbated glucose intolerance and insulin resistance. Such phenomena could not be ascribed to change food intake, since downregulating ADAR1 locally by direct injection of adenovirus to adipose tissue did not show any effect on food intake. Importantly, our data showed that ADAR1 has cell-autonomous effects on insulin sensitivity, and ADAR1 played a role in regulating energy expenditure: local administration of adenovirus to knock down ADAR1 in adipose tissue significantly decreased oxygen consumption rate and CO2 production rate, which may further aggravate the adverse effects on adipocyte expansion and overall obese phenotype. Interestingly, previous publication reported that global knockdown of ADAR1 decreased food intake and ameliorated HFD-induced obesity (Cui et al., 2021). Given the high expression of ADAR1 in the brain, future studies are warranted to dissect the roles and the underlying mechanisms of ADAR1 in regulating food intake.

Minor comments:

1. Please indicate that NC stands for negative control in the text and/or legends.

Response:

Thank you for pointing this out. We have indicated the NC stands for “negative control” throughout the manuscript.

2. Please use the consistent, standard format for gene/protein names throughout the manuscript. The same gene is sometimes all capitalized and sometimes not. For example, PPARg vs Pparg. CEBPb vs C/EBPb vs CEBP/b. etc.

Response:

Thank you very much for your valuable suggestion. We have revised the format of gene/protein names throughout the manuscript according to your suggestion.

3. [The paragraph of ADAR1 overexpression] It is conventional to use the word "transduction" for virus instead of transfection.

Response:

We are sorry for the nonstandard expression. We have carefully corrected the word in the paragraph of ADAR1 overexpression according to the reviewer’s comment. The modification is as follows: For further exploring the influence of ADAR1 on adipogenic differentiation, we overexpressed ADAR1 in MEFs through adenovirus (ad-ADAR1) transduction two days before induction of differentiation.

4. [Fig5 legend] Please correct "...and method".

Response:
Thank you for your careful review. We have corrected the sentence in [Fig.5 legend]. The modification is as follows: Morphology (C), weight (D), and H&E staining (E) of subcutaneous adipose tissue in the control group and overexpressed ADAR1 group.

5. [Fig5 legend] It could be misleading to call adipoq and fabp4 adipogenic genes since they are terminal genes and not the driving factors for adipogenesis

Response:
Thank you for your valuable advice. We have corrected the "adipogenic genes" to "adipocyte marker genes". The modification is as follows: (J-M) qPCR detection of adipocyte marker genes (Adipoq, C/ebpα, Fabp4 and PPARγ) in inguinal adipose tissue in the two groups after 8 weeks of HFD.

6. [Fig7A] Please indicate which band is the target of interest and which more than one band is present.

Response:
Thank you for your careful work. Fig.7A is now referred to as Fig.8A. ADAR1 is ubiquitously expressed with two known isoforms ADAR1L (p150) and ADAR1S (p110) resulting from transcription using alternative promoters and start codons. ADAR1 P110 and ADAR1 P150 are two bands of interest. For TRBP, the band with a higher expression level below is the band of interest.

Hope our amendment can be approved. Thank you.

In the end, we thank you for your precious time to read and review this manuscript. Your comments and suggestion are very valuable for us to further improve our paper. We have carefully understood your opinions and answered and modified one by one. We believe that the revised version can be approved and supported by you for publication, thank you so much!

Second decision letter

MS ID#: JOCES/2021/259333

MS TITLE: ADAR1 inhibits adipogenesis and obesity by interacting with dicer to promote the maturation of miR-155-5P

AUTHORS: Zuying Yu, Ruijie Luo, Yutian Li, Xiaoguang Li, Jiangtong Peng, Kai Huang, and Zhengrui Yang
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.